

XVI. *The Embryology of Monotremata and Marsupialia*.—PART I.

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[PLATES 29–31.]

INTRODUCTION.

IN 1882 the late Professor F. M. BALFOUR suggested my undertaking the study of the development of the peculiar Australian Mammalia and *Ceratodus*. In 1883 I decided to carry out this suggestion, and was elected to the travelling studentship founded in BALFOUR's memory.

The Committee of the Royal Society appointed to administer the Government Grant for the endowment of research gave me a sum of £400 for equipment.

A Sub-committee handed on to me the sum of £100, which they had for a similar object obtained from the same fund.

Through the courtesy of the President of the Royal Society, I obtained letters of introduction from the Colonial Office to the Governors of New South Wales, Victoria, and Queensland, and from the Admiralty to the Commodore of the Australian station.

I arrived in Australia at the end of September, 1883, and for various reasons determined to make Sydney, N.S.W., my headquarters.

I should like to take this opportunity of expressing my thanks to the Ministers of the Colonial Governments, especially to the Right Honourable W. BEDE DALLEY, of Sydney, and to Sir SAMUEL W. GRIFFITHS, of Brisbane, for the assistance and facilities afforded me. The President of the Linnean Society of New South Wales, the Honourable WILLIAM MACLEAY, gave me the use of a temporary laboratory, which I occupied until the Government gave me a more convenient set of rooms in the beginning of 1884. This is only one of many kindnesses for which I am indebted to Mr. MACLEAY.

Guided by Dr. BENNETT's observations, I had intended to go after *Ornithorhynchus* immediately on my arrival, but unfortunately I wasted a fortnight trying to obtain

information in Sydney as to where the animals were to be found in sufficient numbers for my purpose. On October 12 I started for the colder districts in New England, but very soon discovered that no uterine stages were to be obtained during that season. I found, however, that the Marsupial *Phascolarctos* was just beginning to breed, and determined accordingly to collect a series of stages. W. F. GORDON, Esq., kindly invited me to stay with him at Manar, near Lake George, where *Phascolarctos* is exceedingly numerous. In December I also obtained many uterine embryos of *Halmaturus rufus*, as well as a few of other species. I returned to Sydney early in January, engaged a laboratory assistant, M. ÉMILE ROGET, and soon fitted up as a laboratory rooms given me by the Government in Macquarie Street.

During February and March I made short expeditions in search of Marsupial material, and sent home an account of the unique condition of the foetal membranes, hitherto overlooked ('Quart. Journ. Microsc. Sci.', vol. 24, 1884).

Towards the middle of April I went north to the Burnett River to find *Ceratodus*. On the 23rd I obtained males with ripe spermatozoa. Both *Ornithorhynchus* and *Echidna* seemed to be very numerous in the Burnett district; I decided, therefore, to remain there until the Monotreme breeding season was over, in the hope of getting both *Ceratodus* and Monotremes in the same year. The Burnett district presented the further advantage of still possessing a considerable number of black natives. I afterwards found that without the services of these people I should have had little chance of success, and it soon became clear that, in order to work with them, I should have to live under canvas and carry sufficient provisions for an independent camp.

As there were still (May) many weeks of winter to pass before *Echidna* would breed, I visited Gayndah, the only inland township on the Burnett River, with the object of offering a money reward of £10 to anyone who would show me *Ceratodus* spawn. Soon after, I returned to Dalby to purchase a buggy, horses, and camp outfit, and made a flying visit to Sydney to obtain material for a prolonged collecting expedition.

During part of June and July I spent many hours daily in the water, hunting everywhere for the eggs of *Ceratodus*. Towards the end of July the blacks began to collect *Echidna*, and very soon I had segmenting ova from the uterus. In the second week of August I had similar stages in *Ornithorhynchus*, but it was not until the third week that I got the laid eggs from the pouch of *Echidna*. In the following week (August 24) I shot an *Ornithorhynchus* whose first egg had been laid; her second egg was in a partially dilated *os uteri*. This egg, of similar appearance to, though slightly larger than, that of *Echidna*, was at a stage equal to a 36-hour chick. On the 29th I sent in the telegram "Monotremes oviparous, ovum meroblastic" to a neighbouring station, where it would meet the passing mail-man, addressed to my friend Professor LIVERSIDGE, of the Sydney University, asking him to forward it to the British Association at Montreal. Meanwhile I had never relaxed my efforts to find *Ceratodus*; but after four months I was beginning to despair of success. Early in

September I had intended to go further south, so as to continue collecting *Ornithorhynchus*, but almost immediately after making this decision I found the long looked-for eggs of *Ceratodus*. This discovery changed my plan of following *Ornithorhynchus* into colder districts. The exceptional drought of 1884, by reducing the area available for spawning, made the season a particularly favourable one for my work. The next three months were spent in hatching and rearing stages of *Ceratodus*.

During this time the number of my black retainers had increased to upwards of fifty. The women were employed in searching the river weed for *Ceratodus*, while the men collected *Echidna*. It was only occasionally, and then with great difficulty, that I persuaded them to dig for *Ornithorhynchus*. Not only the blacks, but their dogs, refused to eat the animal; *Echidna*, on the other hand, was their favourite food, and their skill in finding them was very remarkable.

The result of this expedition, which had lasted from April to December, was that I had obtained many of the Monotreme stages, and a very complete series of *Ceratodus*; but I recognised that my camp had not been organised on a sufficiently large scale. In order to obtain a complete series of Monotremes, another season was necessary, and a very much larger camp.

My work on the Burnett River was greatly assisted by the hospitality of the squatters. I am particularly indebted to W. F. McCORD, Esq., and GEORGE MUNRO, Esq., who at all times rendered invaluable assistance, both by lending me horses and men, and allowing me temporary supplies from the station stores.

In December I returned to Sydney, with six clear months free for collecting Marsupial embryos, before the Monotremes would begin to breed again. The extreme delicacy of the early stages of Marsupials made it impossible to preserve them perfectly while travelling. The only satisfactory method was to be present at a large Kangaroo drive, where great numbers are driven into a small yard. The Marsupial Acts and the dry seasons had so thinned their numbers that few stations found it profitable any longer to drive the animals; the system of paying so much a head to independent hunters had replaced the yards. Fortunately, through the kindness of some squatters in the Gwydir district, a drive was arranged, and I had the good fortune to get over 80 uterine stages of *Macropus major*, besides several of other species.

From January 1 to the middle of March I travelled in a light buggy, collecting Marsupials. At the end of this time an accident deprived me of the results of this expedition. Crossing the MacIntyre River in a flood, the buggy was upset, and its contents washed away. The two following months were lost through the effects of a fever which I had contracted in the swamps of the Burnett River, and it was not until June that I started to organise the large camp of blacks, to continue the delayed attack on the Monotremes. With the help of my friend, REGINALD BLOXSOME, Esq., over 150 natives were employed during July and August, and they caught between

1300 and 1400 *Echidna* of both sexes, from which a fairly complete series of stages was obtained. A skilful black, when he was hungry, generally brought in one female *Echidna*, together with several males, every day. The former seemed to be much more difficult to find than the latter at this season. The blacks were paid half-a-crown for every female, but the price of flour, tea, and sugar, which I sold to them, rose with the supply of *Echidna*. The half-crowns were, therefore, always just sufficient to buy food enough to keep the lazy blacks hungry. The supplies were carried on a six-horse dray, and the light buggy with four-in-hand proved very much more convenient than the heavier trap of the year before. In September my friend BLOXOME superintended the transfer of the camp to the colder river Mole, further south, where we hoped to dig out the later stages of *Ornithorhynchus* from their nests. I employed some white navvies, who opened up a large number of burrows ; but the renewed exposure in Queensland had brought on my fever again, and this seriously interfered with the completion of the *Ornithorhynchus* series.

The later stages of Monotreme development have, therefore, to be worked out, mainly with *Echidna* material.

PREVIOUS INVESTIGATIONS.

Before entering on the embryology of the Monotremes, it will be, perhaps, interesting to trace the history of opinions held concerning them during the long period of ninety-two years which elapsed from their discovery in 1792 until the complete series of the stages in development were found in 1884. Nearly all the different views held concerning the nature of the female product in Monotremata have been based on indirect evidence, derived from the anatomy of the adult.

SHAW (53 and 54),* who described *Echidna* in 1792, and *Ornithorhynchus* in 1799 (*Platypus*), classed them with Edentata.

BLUMENBACH (13), after studying the skull in 1801, suggested that they might prove to be oviparous (14). Sir EVERARD HOME (35), in the same year, relying chiefly on the absence of mammary glands and the presence of a cloaca, compared them with the ovi-viviparous Sharks and Reptiles. In 1803 M. ÉTIENNE GEOFFROY SAINT-HILAIRE (19) proposed the name "Monotremata," without definitely assigning them a place in the zoological series. Between 1802 and 1819 no new facts were discovered, and in the text-books the animals were classified, either as Mammals, between Edentata and Rodentia (DESMAREST, 1804), or as forming an appendix quite at the end of the Mammalian series (TIEDEMANN, 1808 ; ILLIGER, 1811), or as outside the Mammalia, and "very probably" oviparous (LAMARCK, 1809). In 1812 DE BLAINVILLE (9) pointed out the resemblance to Reptilia, presented by the urino-genital apparatus and the shoulder-girdle ; but he considered the resemblances to Mammalia, and especially to Marsupialia, much more marked. In 1817 CUVIER and OKEN still kept the

* The numbers in parentheses refer to the alphabetical List of Works at pages 480-484.

animals among the Edentata, but used GEOFFROY's term, Monotremata. OKEN suggested that the mammary glands might occupy some unusual position, and so have escaped notice. In 1817, also, Sir JOHN JAMISON (38), writing from Australia to the Secretary of the Linnean Society, described the spur of the male, and stated that the female was oviparous. Sir EVERARD HOME (37), in 1819, discovered that the left ovary alone was functional, and that in the ovary the egg-follicle contained a large quantity of yolk, like a Bird's.

In 1820 Mr. PATRICK HILL, Surgeon, R.N. (34), made some observations which caused him to write to the Linnean Society; the letter was dated January 3, 1821, but was not read until December 18. Mr. HILL describes how he found an "egg in the left ovarium," "of the size of a pea, and of a yellow colour." This specimen was sent to England by a Mr. SCOTT, examined by Sir EVERARD HOME, and afterwards presented to the University of Oxford. This ovarian follicle of HILL's gave rise to considerable misunderstanding; thus FLEMING, in his 'Philosophy of Zoology,' 1822, p. 215, stated that *Ornithorhynchus* eggs had been transmitted to London. Recently, BEDDARD (2) has quoted FLEMING, but omitted to point out that the latter knew that the egg referred to had not been laid, as FLEMING added, "It would be interesting to know the manner of incubation, and whether oviparous or ovo-viviparous." In the 'Isis' for 1823, col. 1425, OKEN, after printing GEOFFROY's remarks, and HILL's letter on which they were based, disposes of the "celebrated history of the *Ornithorhynchus* egg" by pointing out the Mammalian characters of HILL's Graafian follicle. OKEN concludes by saying that the whole story arose from the mammary glands not having been observed.

In 1823, however, MECKEL (40) discovered mammary glands, and his great work (41) was published in 1826. MECKEL, while rejecting both HILL's evidence and OKEN's arguments, pointed out that there was little difference between viviparity and oviparity, since Birds had been artificially made to incubate their eggs in the oviduct. GEOFFROY had by this time become convinced that the animals were oviparous, and he soon denied (22) that MECKEL's glands were mammary. In 1829 GEOFFROY obtained, through Professor GRANT, the egg-shells which had been sent from Australia as veritable *Ornithorhynchus* eggs. GEOFFROY (23) figured the egg, but soon afterwards discovered that it was too large for *Ornithorhynchus*. In 1827 GEOFFROY (22) had stated that the diameter of the pelvis was not more than 5 lines. The reputed egg measured $1\frac{3}{8}$ inch \times $\frac{6}{8}$ inch. GEOFFROY's first hypothesis, 1827 (22), was that the egg was formed in the cloaca, but so rapidly as not to cause a serious obstruction! His second, 1833 (27), was that the egg remained in the oviduct until hatching, because it could not pass through the small pelvis.

In 1832 the first direct evidence bearing on the question was published in the 'Proceedings of the Committee of Science and Correspondence of the Zoological Society of London.'

Lieut. the Hon. LAUDERDALE MAULE (39) wrote to his friend Dr. WEATHERHEAD

that he had tried to discover the grounds for the generally-accepted belief that *Ornithorhynchus* both laid eggs and suckled its young. He found in females "eggs of the size of a musket-ball, but without the hard outer shell, and, in the nest, young ones, and remains of a substance resembling egg-shell."

Three young *Ornithorhynchi*, found by Lieut. MAULE in the nest, were sent home to Dr. WEATHERHEAD; two of these were given to Sir R. OWEN, the third was given to M. GEOFFROY. Sir R. OWEN (45) discovered in these the caruncle on the bill. This striking structure, however, only confirmed OWEN in his belief in ovi-viviparity. He wrote, 1834,* the Marsupialia and Monotremata "may be regarded as an aberrant group of Mammalia, characterised by an ovo-viviparous generation."

Sir R. OWEN's researches were made possible by the energy of Dr. GEORGE BENNETT, who himself made several expeditions with the object of finding the early stages of *Ornithorhynchus*. Dr. BENNETT (3, 4, 5, and 6) has accumulated a large number of interesting observations on the habits of the animal, and has never ceased trying to furnish Sir R. OWEN with the material necessary for solving the problem of Monotreme generation.

The next direct observation was made in 1847 by M. JULES VERREAU (55) in Tasmania. He writes:—"Le nombre d'Ornithorhynques que j'ai possédé m'a parfaitement démontré que cet animal ne pond pas d'œufs, mais qu'il est ovo-vivipare."

Sir R. OWEN (47) reviewed M. VERREAU's paper in 1848, and stated that he had calculated the period of uterine gestation at about six weeks! In a foot-note, p. 318, Sir R. OWEN, contradicting a statement in CARPENTER'S 'Human Physiology,' to the effect that there was no positive evidence in favour of the ovi-viviparity of Monotremes, says, "The minute size of the ovarian ovum, and consequently of the vitellus; the presence of small ova, with a delicate chorion and without chalazæ or shell, in the uterine portion of the oviduct; the absence of any shell-forming portion of the oviduct,—all are elements of a body of positive evidence in favour of the ovi-viviparity of the *Ornithorhynchus*, which needs only the discovery of the foetus *in utero* for decisive confirmation."

In 1864 Mr. GEORGE RUMBY (52), a gold-receiver in Australia, obtained from some miners a living female *Ornithorhynchus*. This animal, shut up in a gin-case, laid two eggs which were "white, soft and without shell, easily compressible, and about the size of a crow's egg." Mr. RUMBY wrote in a letter that these eggs might be abortions caused by fear, and this suggestion was evidently accepted by OWEN, who published the letter in 1865 (48). These eggs were also seen by Dr. JOHN NICHOLSON (42), who wrote directly to OWEN about them (48). Sir R. OWEN in this paper, 1865 (48), describes a mammary foetus sent to him by Baron FERDINAND VON MUELLER and Dr. RUDALL, and, in discussing the uterine ova, describes the "smooth chorion as being firmer than that of Rodentia; whence, and for other reasons (*vide Phil. Trans.*, 1834)," he still considered the animals ovi-viviparous.

* 'Phil. Trans.', 1834, p. 356.

From 1875 to 1883 Dr. GEORGE BENNETT's son (7, 8) collected material for Sir R. OWEN in Queensland. In 1880 Sir R. OWEN (49) described and figured several ova of *Echidna*, taken from the uterus. In the largest of these, measuring 6 mm. in diameter, he observed an artificially-produced furrow, which he described as the first furrow of segmentation, mentioning this fission of the germ as "additional evidence of the viviparity*" of the Monotremes."

In 1882 Sir R. OWEN found, lying free in the uterus of an *Echidna*, a large ovum with a "thick chorion"; *vide* paper published in 1884 (50). Mr. HAACKE (32, 33) found, on the 25th of August, 1884, an egg-shell, the remains of a rotten egg, in the pouch of *Echidna*, and showed it to the Royal Society of South Australia on the 2nd of September.

PART I.—THE EGG MEMBRANES, AND THE OVA UP TO THE FIRST STAGES OF SEGMENTATION.

Part I. of my studies contains an account of the egg membranes, and the development of the ovum up to the first stages of segmentation, in Monotremata and Marsupialia. This limitation will enable me to trace the gradual replacement of ovarian food-yolk by uterine nutrition through the Mammalia.

The comparison of the egg membranes yields a striking phylogenetic interpretation of their development.

The few previous investigators have failed to trace the egg membranes.

Sir EVERARD HOME, Mr. HILL, Sir R. OWEN, and M. VERREAUx have described the appearance of the ovary in Monotremata. The two first observers pointed out the large size of the Graafian follicle, and Sir EVERARD (37) figured the yellow yolk they contained, but Sir R. OWEN, in 1834 (44), still considered it probable, on *a priori* grounds, that the Monotremes had a small ovum, like other Mammalia.

More recently POULTON (51), BEDDARD (2), and GULDBERG (31) have observed the large size of the ovarian ovum, the eccentric position of the germinal vesicle, and the fact that the ovum completely fills the follicle during the whole period of ovarian life.

POULTON and GULDBERG have wrongly stated that the follicular epithelium remains always a single layer of cells.

BEDDARD figures the true condition, but agrees with the other two observers, ascribing the appearance of his preparation to bad preservation. POULTON came to the conclusion that it was probable that segmentation would be found to be unequal, perhaps partial. GULDBERG was unaware, when he wrote, that I had already described the partial segmentation. GULDBERG and BEDDARD lost all trace of the vitelline membrane in late stages. The former says the follicular epithelium becomes changed into a chorion:—"es scheint, als ob die Zellen verschmolzen sind, um eine helle, schwach tingir-

* I have added the italics, because the quotation shows the value of the old terms "ovi-viviparous" and "viviparous."

bare Membran zu bilden." This statement is of interest in connection with SELENKA's (57) similar account of the formation of an egg membrane in the Opossum. The ovary of Marsupialia has been described and figured in several genera. Sir R. OWEN observed the enormous stalked follicles of *Phascolomys*. POULTON's account of the development of the Graafian follicle in *Phalangista* is the only paper on the subject known to me. He noticed that the cumulus prolierus separated from the wall of the follicle. SELENKA (57) found follicular cells in the Fallopian tube of the Opossum. Observations on the uterine egg membranes of Monotremata have been made by Sir R. OWEN, who in 1865 distinguished two membranes, an outer "chorion" and an inner "membrana vitelli," but he wrongly stated that the "increase of the size of the uterine over the ripe ovarian ovum was due to an increase of fluid between the chorion and the membrana vitelli." SELENKA is the only observer of the early uterine stages of the egg membranes of Marsupialia. He derives the outer membrane of the uterine ovum directly from the follicular epithelium, and further states that the zona pellucida soon disappears, which results in his confusing the albumen layer of the early stages with the coagulum surrounding the embryo in later stages. SELENKA, in his preliminary note on the Opossum (56), says, "Die Eier halten die Mitte zwischen den meroblastischen und holoblastischen." The exact meaning of this statement is made clear by the beautiful drawings in his Memoir.

A.—THE EGG MEMBRANES.

1.—*Monotremata*.

i. *In the ovary.*—Round the very young ova a fine membrane is present between the single row of follicular cells and the ovum. I shall speak of this as the vitelline membrane, but whether it is produced by the ovum or the follicular epithelial cells I have not attempted to determine.

With the growth of the ovum the vitelline membrane increases enormously in thickness. In ova of .32 mm. diameter it reaches its maximum thickness of .016 mm. With the formation of the yellow yolk spheres the vitelline membrane again becomes thinner, till in the ripe ovarian ovum it has no longer a measurable thickness. The vitelline membrane is perforated by protoplasmic processes connecting the protoplasm of the ovum with that of the follicular epithelium.

Round very young ova the follicular epithelium consists of a single layer of flattened cells, each of which, like the ovum itself, consists of clear protoplasm nearly free from granules.

The difference between a ripe ovum and such an unstainable cell is caused by the addition of food material, which is formed in the cells of the follicular epithelium as well as in the ovum itself, and appears first as minute granules in the neighbourhood of the nuclei and germinal vesicle. Every stage is to be found, from the most minute granules up to the largest yellow yolk spheres of the ripe ovum.

From the nucleus of each follicular cell, and from the germinal vesicle, streams of these yolk granules travel into the body of the ovum. The result of this double mode of origin of the yolk is that the yolk is interrupted at one place, viz., where the germinal vesicle lies. The yolk in Monotremata has the same arrangement as that of Birds, where a central bottle-shaped mass of smaller spheres is continued from the germinal disc to the centre of the ovum. The streams of yolk granules passing into the ovum, immediately on entering, are more or less parallel to each other, and give a radially striated appearance to the peripheral layer of the ovum (Plate 29, fig. 1). A similar appearance in other Vertebrate eggs has been described as a definite membrane, and named the *zona radiata*. The time during which the greater number of yolk granules are formed in the cells of the follicular epithelium corresponds with a marked change in the appearance of the layer itself. When the ovum has reached a diameter of .2 mm. the cells of the follicular epithelium, already much more columnar than in the younger stages, divide rapidly and form a layer three to four cells deep (Plate 29, fig. 1, *fe.*). When the ovum has increased to .5 mm. diameter it has received from the follicular cells such a number of yolk granules as would be nearly sufficient, if the granules were swollen to yellow yolk spheres, to completely fill a ripe ovum measuring 3 mm. in *Echidna* and 2.5 mm. in *Ornithorhynchus*. Between the stages of .32 mm. diameter and .5 mm. diameter the follicular epithelium again becomes one cell deep. This change corresponds with the change in the manner of growth of the yolk. Up to stage .32 mm. the formation of new granules was the chief function of the epithelium. After the granules are formed the absorption of fluid by osmosis becomes relatively more important; consequently, the yolk granule forming period may be conveniently spoken of as the first period of the activity of the follicular epithelium, while the period of absorption of fluid may be described as the second period.

Both processes, of course, go on during the whole of both periods. Each period includes the maximum activity of one of the two processes, granule formation or absorption of fluid.*

The active change of granules into yellow yolk spheres by the absorption of fluid is marked by the return of follicular epithelium to the condition of a single row of cells.

Plate 29, fig. 2, shows the condition of the follicular epithelium (*fe.*) and the vitelline membrane (*vm.*) in an ovum measuring 1 mm. diameter.

The follicular epithelium becomes so flat that BEDDARD (2), who has recently described the ovary of *Echidna*, failed to find it; while GULDBERG, as has been already mentioned, stated that the follicular cells fused together to form a clear "chorion."

When the ovum has reached its maximum diameter the follicular epithelium again wakes up; and the cells increase enormously in size, and each nucleus becomes larger than an entire cell in the previous stage, Plate 29, fig. 3 (*fe.*). This renewed activity

* It is to be understood that this account of the origin of yolk is only a description of the structural appearances presented by preserved material. The physiological changes may be much more complicated.

of the follicular epithelium constitutes a well-marked stage, and will be described as the third period.

The follicle has by this time come to project from the ovary so much that, in many cases, there is a marked pedicle of attachment. The follicular epithelium, beginning in the region underlying the projecting surface of the follicle, divides rapidly, and the cells, becoming enormously enlarged, secrete a dense homogeneous substance on their inner face, next the vitelline membrane (Plate 29, fig. 4, *ch*). This process of secretion goes on until the whole egg is suspended in a dense layer, which, from its fate, I shall speak of as the "pro-albumen." The follicle, being now clasped by the open mouth of the Fallopian tube, bursts, and the egg is received into the Fallopian tube.

A few cells of the follicular epithelium remain attached to the pro-albumen. The majority of the follicular cells remain behind, inside the follicle, and there they multiply so rapidly that very soon the whole cavity of the follicle is occupied by gigantic cells derived from continued division of the follicular epithelium, between which connective tissue cells have also grown in from the walls of the follicle itself. The further changes that take place in this "corpus luteum" need not be described.

ii. *In the Fallopian tube*.—I found one female *Ornithorhynchus* with two eggs in the dilated end of the Fallopian tube (infundibulum). Both eggs had begun to segment, and one had already acquired eight segmentation nuclei. Each egg was enclosed by its thin vitelline membrane (Plate 30, fig. 1; Plate 31, fig. 3, *vm.*), and surrounded by the pro-albumen. Here and there traces of the follicular cells, which remained attached to their own secretion, are visible in the sections. A few darkly staining granules were visible all round the ovum, connected with each other by a fine, darkly staining line, just inside the vitelline membrane. This layer was already visible in some sections through ripe ova, after the pro-albumen was formed. The unsegmented egg of *Echidna* (a medium section through the germinal disc of which is figured on Plate 31, fig. 1) was taken from the lower part of the Fallopian tube. In this egg the vitelline membrane has already increased in thickness to about .0016 mm., and the pro-albumen has also increased in thickness until it becomes the definite albumen layer by absorption of fluid in the Fallopian tube. In the living egg this albumen has the same appearance as the albumen of a Hen's egg, and, treated with alcohol, it comes down as a granular precipitate. (Plate 30, fig. 3, *alb.*; Plate 31, fig. 1, *alb.*) The albumen in hardened ova varies in thickness at different places by reason of its fluid nature. That the section figured in Plate 30, fig. 3, happens to have less thickness of albumen than the pro-albumen from which it arose is explained in this way.

Outside the albumen there is now a new structure—the shell membrane (Plate 31, fig. 1, *sh.*)—which first appears in the lower part of the Fallopian tube. The shell membrane is of a tough parchment-like consistency, and does not stain with hæmatoxylin or borax carmine. On the outside it has a roughened surface, which in section (Plate 31, fig. 1, *sh.*) is seen to be caused by the presence of numerous fine

villi. I have not yet tried to trace the deposition of the shell to any special glands. From the fact that these villi become longer as the egg increases in size it is clear that the shell does not thicken at the expense of the albumen layer inside.

iii. *In the uterus.*—The egg, arrived in the uterus, has already received its full complement of membranes. The albuminous investment and the shell have added 1·5 mm. to the egg's diameter. The egg continues to increase in diameter after its arrival in the uterus, and by the time it has reached a diameter of 6·5 mm. the albumen layer has entirely disappeared, and the vitelline membrane thus comes to lie close to the shell. (Plate 30, figs. 3 and 4.) Meanwhile both shell membrane and vitelline membrane have again increased in thickness. (Plate 30, fig. 2, *sh.* and *vm.*) Another layer, which stains darkly, present in the ovary after the pro-albumen appeared as the very delicate line containing granules, already described, lies inside the vitelline membrane. I look on it as a coagulum formed by reagents from the nutritive fluid entering the ovum. In the uterus it becomes very conspicuous on the disappearance of the albumen, and its presence enables the opening of the blastopore to be traced.

The egg, on leaving its follicle, measured 2·5 mm. to 3 mm. in diameter. When it is laid it measures 15 mm. by 12 mm.*

This enormous increase is due to the absorption of fluid from the walls of the uterus. The process shows itself by the continually increasing quantity of darkly staining coagulum inside the vitelline membrane and through the body of the yolk. Up to the close of segmentation this layer is thicker over the blastoderm than elsewhere. In many sections mounted in balsam the line between the vitelline membrane and this coagulum is not distinct, and the two appear as one thick vitelline membrane.

The fluid layer betrays its nature, however, by passing in between the cells, and into an opening of the blastopore.

The earliest stage of the shell membrane has been already described. Some of the stages it passes through before being laid are shown on Plate 30 (figs. 2, 3, and 4, *sh.*). In *Echidna* I have not detected any calcic salts in the shell after laying, but on treating the shell of *Ornithorhynchus* with dilute hydrochloric acid a considerable quantity of gas is given off. When fresh-laid, the egg has a thickness of 5 mm. and is of an opaque white colour; the cones figured on Plate 30 (fig. 4, *sh.*) are directly derived from the fine villi on the outside of the young shell membrane.

2.—*Marsupialia.*

i. *In the ovary.*—The development of the membranes just traced in Monotremata proceeds in exactly the same way in *Phascolarctos cinereus* up to the stage when the yolk granules begin in the Monotremata to become the characteristic yellow

* The laid eggs of both *Echidna* and *Ornithorhynchus* vary somewhat in size. I have a normal *Echidna* egg as small as 13 mm. by 12 mm.

yolk spheres (compare Plate 29, fig. 1, with Plate 29, fig. 5). In *Phascolarctos*, as in Monotremata, the delicate membrane surrounding the youngest ovum gradually changes into a distinct and strong membrane surrounding the ripe ovum. The ripe ovum of *Phascolarctos*, measuring .17 mm., resembles an ovum of *Echidna* or *Ornithorhynchus* measuring .25 mm. The follicular epithelium throughout the ovarian period is connected with the ovum by numerous processes perforating the vitelline membrane, along which the yolk granules pass into the ovum.

The youngest ova of *Phascolarctos* exactly resemble the youngest already described in Monotremata. The single layer of follicular cells soon becomes columnar, and also, by division, several rows deep. A cavity appears between the cells, in the same way as in Placentalia, and soon a typical Graafian follicle with its "discus proligerus" is formed.

The follicle of *Phascolarctos* grows very much larger than in most Marsupialia. In *Phascolarctos* the ripe follicle is in many cases attached to the ovary by a very slender pedicle. The follicle is elliptical in shape, and measures 10 mm. by 7 mm. It is thus enormously larger than the ripe follicle of Monotremata.

The split in the layers of the follicular epithelium, which forms the cavity of the Graafian follicle, extends completely round the ovum, so that the ovum, still surrounded by the follicular epithelium several cells deep, now lies in the centre of the follicle, with the so-called "liquor folliculi" on all sides of it. The "liquor folliculi" contains numerous branched cells, connecting the epithelial lining of the follicle with that surrounding the ovum (Plate 29, fig. 5, *fe.*).

ii. *In the Fallopian tube.*—I have no observations on the membranes in the Fallopian tube of Marsupials. The few young ova I obtained were used for other purposes. I was not aware of the existence of the pro-albumen at the time I found them.

iii. *In the uterus.*—The ovum of *Phascolarctos* passes rapidly into the uterus, and the ovum figured on Plate 29, fig. 5, had only reached the stage of the first furrow of segmentation. In this ovum the same membranes are present as in the corresponding stage of *Echidna*, with the striking difference that the follicle cells are still attached to a dense layer, similar to the pro-albumen in the Fallopian tube of Monotremata. This layer (Plate 29, fig. 5, *ch.*) lies immediately outside the vitelline membrane. Imbedded in it, and lying on the outside of it (Plate 30, fig. 5, *fe.*), are a large number of follicular cells, and, although their nuclei and nucleoli are still present, and suffice to prove their cellular nature, their general appearance, spherical form, and the absence of connecting processes between the cells, show that they are breaking down.

Though I have failed to trace the formation of this layer in Marsupials from the follicular epithelium, its structure, position, and history all point to its being homologous with the pro-albumen of Monotremata. Towards the outside the cells and the pro-albumen are surrounded with fluid (Plate 30, fig. 5, *alb.*), and the outer part of the pro-albumen itself is less dense than the inner.

Enclosing the whole egg is a thin transparent membrane .0015 mm. thick, similar

to the shell membrane of Monotremata (Plate 30, fig. 5, *sh.*). The further changes undergone by these membranes enclosing the ovum are as follows:—The ovum soon increases in size, and in an ovum measuring 3 mm. the shell has become markedly thicker, viz., 0.1 mm. The vitelline membrane also increases slightly in thickness, but the great increase in size of the ovum is due to the swelling up of the follicular secretion by the absorption of fluid from the walls of the uterus (Plate 30, fig. 7, *abb.*). Two stages are shown in section of this swelling up (Plate 30, figs. 6 and 7). All traces of follicular cells have vanished by this time. The further changes that take place with the formation of the blastodermic vesicle and development of the yolk sac are similar to those that occur in Monotremata at a corresponding age. The albumen soon disappears, and the vitelline membrane comes to lie close to the shell. No sooner is the albumen layer formed than it begins to pass through the vitelline membrane to nourish the ovum, and after the albumen layer has disappeared another layer has become very conspicuous inside the vitelline membrane. This layer is a coagulum formed from nutritive fluid on its way to feed the developing embryo, and shows its fluid nature, in the same way as pointed out in Monotremata, by passing into the open portion of the blastopore, and between the cells of the blastoderm. The distinction between the vitelline membrane, now considerably increased in thickness, and this coagulum is difficult to trace all round the ovum.

This fact has led SELENKA to describe the vitelline membrane as disappearing at an early stage, and the albumen as being directly continuous in development with the coagulum. The membrane, composed of the shell and the vitelline membrane, persists up to the stage when the blastodermic vesicle becomes fixed to the walls of the uterus. It is possible that it may persist for a longer period over the non-vascular unattached area of the yolk sac and allantois (*vide* my paper, 'Quart. Journ. Microsc. Sci.', 1884). The blastodermic vesicle enclosed in the membrane just described, measuring 15 mm. in diameter, floats freely in the uterus, and is exactly comparable to the laid egg of Monotremes. I have not found any trace of villi on the surface of the shell in Marsupials.

B.—THE DEVELOPMENT OF THE OVA UP TO THE FIRST STAGES IN SEGMENTATION.

Monotremata and Marsupialia.

Some of the changes that take place in the ovum have been described in dealing with the follicular epithelium. The ovum, while maintaining its character of a single cell, has become loaded with food material, which has been deposited in a horse-shoe-shaped mass round the germinal vesicle. This horse-shoe-like arrangement of the yolk is caused by its double mode of origin, and is common to all Vertebrata with yolk-forming follicular epithelium. The different positions in which the yolk has been described as arising in meroblastic eggs may not be so different as has been supposed. At the end of the first period in Monotremata the yellow yolk spheres

appear as such at considerable distance from the surface of the ovum. In the ripe egg there is only a very thin layer of fine granules and white yolk, between the yellow yolk and the vitelline membrane. This distribution appears to be due to the relative rapidity the formation of yolk granules bears to their absorption of fluid at any one period. The active protoplasm of *Phascolarctos*, like that of Monotremata, is aggregated to one pole of the ovum. There is a lens-shaped germinal disc in *Phascolarctos* as in Monotremata (Plate 29, fig. 5, and Plate 31, fig. 1).

The yolk of *Phascolarctos* never gets beyond the stage of white yolk. The white yolk immediately below the germinal vesicle in Monotremata is the only part of the ovum where oil (?) globules occur (Plate 31, fig. 1). The whole yolk of *Phascolarctos* is similar to the region below the germinal disc of Monotremes. The details of the segmentation and the exact behaviour of the cells to form the gastrula will be traced in Part II. I shall here only describe the general characteristics of segmentation in Monotremata and Marsupialia, and point out how the segmentation of Placentalia has been derived from this. The first furrow marks out the germinal disc into a larger and a smaller area (Plate 30, fig. 5, n_1 and n_2). The first furrow has therefore made the ovum bilaterally symmetrical. The second furrow appears at right angles to the first, and divides the germinal disc into four regions, two larger and two smaller (Plate 31, fig. 2, n_1 , n_2). All these regions are connected with each other and with the yolk by protoplasmic processes passing across the furrows. These first four nuclei give rise by division to all the nuclei of the future embryo. No nucleus of the yolk which would explain spontaneous formation of "yolk nuclei" is present. So far the description applies equally to *Phascolarctos* and to Monotremata, but the ovum of *Phascolarctos* is no larger than that of Placentalia.

That the first two segmentation furrows should fail to divide the ovum, shows that although the ovum has nearly regained its original alecithal condition, it still retains the secondary arrangement of protoplasm, induced by the yolk of its more immediate ancestors.

Comparison of the Egg Membranes of Monotremata and Marsupialia with those of Placentalia.

From the detailed account of the development of the egg membranes in Monotremata and Marsupialia (*Phascolarctos*) it is clear that there are two primary egg membranes in each group, the vitelline membrane and the pro-albumen, while a secondary egg membrane, the shell membrane, is added in the Fallopian tube and uterus.

There is a strong presumption that the egg membranes of Placentalia are homologous with those of Monotremata and Marsupialia. I shall attempt to show that the three membranes I have found in Monotremata and Marsupialia are present in Placentalia.

There are two egg membranes generally recognised in Placentalia, the zona pellucida and the vitelline membrane.

This vitelline membrane was first noticed by VALENTIN; BARRY (60) figured it in 1838, but BISCHOFF (66) immediately afterwards explained that BARRY and the previous observers had been misled by a line caused by the existence of cilia round the young ovum.

H. MEYER (72), REICHERT (73), and VAN BENEDEK (63) have distinguished this structure, and regarded it as a true vitelline membrane. HEAPE (70) found and figured it in sections of the ripe ovum of the Mole.

BARRY stated in his papers that it disappeared by liquefaction, but in a foot-note in p. 338 in his last paper he says that it "may perhaps contribute to the thickening" of the zona pellucida; and this foot-note of BARRY'S is evidence confirmatory of the suggestion that I now make concerning this membrane.

VAN BENEDEK (64) says the appearance of this true vitelline membrane coincides with maturation and the formation of polar bodies.

I would suggest, therefore, that it is the homologue in Placentalia of the layer which, appearing during maturation as a line containing granules, becomes the coagulum of later stages in Monotremata and Marsupialia.

VON BAER (58) discovered the zona pellucida in the ovary. BARRY (62) figured its radial striation, caused by the pointed ends of the follicular epithelium, and its granular outer layer.

In 1854 REMAK described the radial striation, but it was not until 1870 that WALDEYER (74) called attention to the distinction between the outer and the inner layers of the zona radiata.

WALDEYER considered the granular layer a product of the follicular epithelium. BALFOUR (59) put forward a curious hypothesis with regard to this outer layer. He considered that it was the remains of the vitelline membrane surrounding young ova. BALFOUR'S hypothesis was rendered necessary by his comparison of the inner zona radiata of Mammalia with the transitory appearance which he called zona radiata in Elasmobranchs.

VAN BENEDEK (65), p. 514, pointed out BALFOUR'S mistake, and said, "It is certain that the membrane which BALFOUR called vitelline membrane in the Rabbit is the zona radiata, and not its outer granular layer." There is, therefore, in Placentalia, as in Monotremata and Marsupialia, a delicate membrane round the young ovum, which becomes the thick membrane known as the inner homogeneous layer of the zona pellucida. This membrane is the true vitelline membrane of Placentalia. The outer granular layer may therefore be interpreted as the homologue of the pro-albumen.

The outer covering of the uterine embryo was discovered by DE GRAAF in 1692. VON BAER (58) suggested that this "villous chorion" (*Schalenhaut*) was directly derived from the zona pellucida of the ovary.

COSTE and RUDOLF WAGNER held the same view; but PURKINJE, VALENTIN, and ALLEN THOMSON all considered that the chorion probably arose in the oviduct, like the egg-shell of other Vertebrates.

WHARTON JONES (71) was the first to notice the addition of the albuminous coating in the Fallopian tube. He considered that this coating gave rise to the chorion. BARRY (61) has given a most elaborate account of the origin of the chorion. His results are strikingly similar to what I have described in *Phascolarctos*; and it is not too much to admit that, if no one had thrown doubts on BARRY's work, I should have had no difficulty in comparing the membranes of *Phascolarctos* with those of Monotremata and Marsupialia. Dr. BARRY (62) stated that the chorion arose from "cells" (not the cells of the tunica granulosa, but "cells" appearing in the Fallopian tube) on the outside of the zona pellucida; that this chorion imbibed fluid, and separated from the zona pellucida; that after the fluid was absorbed the zona pellucida again lay close to the chorion; and that the chorion gave rise to villi. Dr. BARRY's experiment of crushing the fresh ovum, and finding that its contents, passing outside the zona pellucida, were still contained in his chorion, is striking evidence in favour of the existence of such a membrane, although, unfortunately, no recent observers have described any trace of it.

TABLE showing the Homologies of the Egg Membranes in Mammalia.

	MONOTREMATA.	MARSUPIALIA.	PLACENTALIA.
Layer of coagulated fluid in	= Coagulum . . .	= Coagulum . . .	= Vitelline membrane of hardened mature ova.
Egg membranes.			VAN BENEDEK.
Primary.	Vitelline membrane = Vitelline membrane = Vitelline membrane = Zona radiata = inner layer of mature ovary, zona pellucida.		
Secondary.	Pro-albumen secreted = Pro-albumen . . . = Pro-albumen . . .	Outer granular layer of zona pellucida (WALDEYER).	
	by follicular epithelium.		
	Shell membrane = Shell membrane = Shell membrane = Villous? "non-cellular" formed outside albumen in Fallopian tube and uterus.	with villi which become "cones."	chorion (BISCHOFF).
		without villi?	

Comparison of the Egg Membranes of Mammalia with those of other Vertebrata.

Very numerous statements have been made concerning the egg membranes of both Ichthyopsida and Sauropsida (68 and 69). I shall not attempt to compare all the membranes that have been described, because it is both certain that many are either transitory arrangements of the peripheral protoplasm of the egg or the product of hardening reagents, and also probable that some are optical illusions.

That a pro-albumen such as I have described in Monotremata has not been recognised in most Vertebrata is due possibly to the shortness of the period of its formation.

CUNNINGHAM (67), however, has recently shown that such a structure exists in *Myxine*, but he assumed that it gave rise directly to the shell, and consequently could

not be homologous with the albumen or the shell of *Elasmobranchii*. It seems more probable that the difference between the shell of *Elasmobranchii* and that of *Myxine* is due to the fact that in *Elasmobranchii* the pro-albumen absorbs fluid in the course of its passage down the oviduct.

The shell membrane presents another difficulty. I have shown that in Marsupialia it does not increase at the expense of the albumen.

CUNNINGHAM states that the shell of *Myxine* arises directly from the pro-albumen, and others have imagined a similar origin for it in *Elasmobranchs* and *Sauropsida*.

VON BAER compared the albumen of a Frog's egg to the shell of a Bird's.

These considerations show that further investigations are necessary in both *Ichthyo-psida* and *Sauropsida* before the three membranes can be traced through Vertebrates.

SUMMARY.

In Monotremata and Marsupialia—

- I. There is a vitelline membrane which, appearing between the young ovum and the follicular epithelium, persists until hatching in Monotremata, and until late uterine stages in Marsupialia.
- II. There is a second primary egg membrane secreted by the follicular epithelium shortly before the ovum leaves the ovarian follicle—the pro-albumen.
- III. The pro-albumen, by absorption of fluid in the Fallopian tube and uterus, becomes the albumen layer outside the vitelline membrane.
- IV. A secondary egg membrane—the shell membrane—is found in the Fallopian tube, and becomes thicker in the uterus.
- V. The albumen soon disappears, and the vitelline membrane comes to lie next the shell.
- VI. The ovum absorbs fluid from the uterus, and increases in Monotremes from about 3 mm. to 15×13 mm.
- VII. A layer, simulating the appearance of a membrane, inside the vitelline membrane, is a coagulum formed by reagents from the nutritive fluid passing into the ovum.
- VIII. There is a germinal disc, and the ovum undergoes a partial segmentation in Monotremes and *Phascolarctos*.

In Placentalia—

- IX. The vitelline membrane has generally been known as part of the zona pellucida.
- X. The pro-albumen is probably represented by the outer "granular layer" of the zona pellucida.
- XI. The shell membrane has not been recognised in its early stages, except by BARRY. The "villous chorion" (non-cellular, BISCHOFF) is probably partly derived from a true shell membrane.

XII. The delicate layer immediately surrounding the ripe ovum, known as the "true vitelline membrane," VAN BENEDEK, is perhaps the first stage of the substance described as "coagulum" in Monotremata and Marsupialia.

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EXPLANATION OF THE PLATES.

Reference Letters.

alb = albumen.
b = basilar membrane of follicular epithelium
bl = posterior opening of blastopore.
ch = pro-albumen.
ep = epiblast.
fe = follicular epithelium.
gv = germinal vesicle.
hy = hypoblast.
o = oil (?) globule.
*n*₁ = nucleus of smaller segmentation area.
*n*₂ = „ larger „ „ „
sh = shell membrane.
*sh*₁ = middle layer of shell membrane.
*sh*₂ = papillæ of shell membrane.
um = coagulum.
vm = vitelline membrane.
*y*₁ = yolk granules.
*y*₂ = white yolk.
*y*₃ = yellow yolk.
zr = zona radiata.

PLATE 29. ZEISS, oc. 2, obj. $\frac{1}{18}$ homogen. : cam. luc.

Fig. 1. *Echidna*.—Small portion of a section through the ovarian ovum, measuring 32 mm. in diameter. 1st period: *fe*, follicular epithelium; *vm*, vitelline membrane; *zr*, zona radiata; *y*, yolk granules; *y₂*, white yolk; *o*, oil globule; *b*, basilar membrane.

Fig. 2. *Echidna*.—Small portion of a section through the ovarian ovum, measuring 1 mm. in diameter. 2nd period: lettering as in fig. 1.

Fig. 3. *Echidna*.—Small portion of a section through the nearly mature ovarian ovum. Beginning of 3rd period: lettering as in fig. 1.

Fig. 4. *Echidna*.—Small portion of a section through the ripe ovarian ovum, measuring 3 mm. in diameter. 3rd period: *ch*, pro-albumen.

Fig. 5. *Phascolarctos cinereus*.—Medium section through a nearly mature ovarian ovum taken from the "liquor folliculi" of a follicle measuring 9 mm. \times 6 mm.: *fe*, follicular epithelium; *vm*, vitelline membrane; *gv*, germinal vesicle.

PLATE 30. ZEISS, oc. 2, obj. $\frac{1}{18}$ homogen. : cam. luc.

Fig. 1. *Ornithorhynchus*.—Small portion of a section through the segmenting ovum, taken from the open end of the Fallopian tube, measuring 2.6 mm. in diameter: *ch*, pro-albumen; *vm*, vitelline membrane.

Fig. 2. *Echidna*.—Small portion of a section through the segmenting ovum taken from the uterus, and measuring 6 mm. in diameter: *sh*, shell; *alb*, albumen; *vm*, vitelline membrane; *um*, coagulum; *bl*, blastopore; *ep*, epiblast; *hy*, hypoblast.

Fig. 3. *Ornithorhynchus*.—Small portion of a section through the segmenting ovum taken from the uterus, and measuring 6 mm. in diameter: *sh*, base of shell; *sh₁*, middle layer of ditto; *sh₂*, papillæ of ditto.

Fig. 4. *Echidna*.—Small portion of a section through the blastodermic vesicle, taken from the uterus, and measuring 9 mm. in diameter: *sh₂*, cones derived from papillæ of previous stage.

Fig. 5. *Phascolarctos cinereus*.—The 17th section of a vertical longitudinal series of 35 sections through the segmenting ovum, containing 2 nuclei, taken from the uterus, and measuring 17 mm. in diameter: *sh*, shell membrane; *fe*, cells of follicular epithelium; *alb*, albumen; *ch*, pro-albumen; *vm*, vitelline membrane; *y₁*, protoplasm, with finest yolk granules; *y₂*, white yolk; *n*, nucleus of smaller segmentation area; *n₂*, nucleus of larger segmentation area.

Fig. 6. *Phascolarctos*.—From uterus, 28 mm. in diameter, stage of 4 nuclei: *sh*, shell; *ch*, pro-albumen; *alb*, albumen; *vm*, vitelline membrane.

Fig. 7. *Phascolarctos*.—From uterus, 31 mm. in diameter; lettering as in fig. 6.

Fig. 8. *Hypsiprymnus*.—From uterus, 4 mm. in diameter: *sh*, shell membrane; *vm*, vitelline membrane; *um*, coagulum; *bl*, blastopore; *ep*, epiblast; *hy*, hypoblast. (*Cf.* fig. 2.)

PLATE 31. ZEISS, oc. 2, obj. c. : cam. luc.

Four sections through the germinal disc of *Echidna* and *Ornithorhynchus*. The violet corresponds to the distribution of the more active protoplasm. The yellow yolk spheres are coloured buff.

Fig. 1. *Echidna*.—Median section through the germinal disc of an unsegmented ovum taken from the lower part of the Fallopian tube, measuring 3.2 mm. in diameter: *sh*, shell; *alb*, albumen; *vm*, vitelline membrane.

Fig. 2. *Echidna*.—Stage of four segmentation nuclei. Lateral section through one pair of nuclei made in a plane at right angles to the first furrow; ovum 4.5 mm. in diameter: *n*₁, nucleus of smaller area; *n*₂, nucleus of larger area. Other letters as in fig. 1.

Fig. 3. *Ornithorhynchus*.—From open end of Fallopian tube, 2.6 mm. diameter. Stage of eight segmentation nuclei: *ch*, pro-albumen.

Fig. 4. *Echidna*.—From uterus, 5 mm. in diameter. Median vertical longitudinal section through germinal disc: *um*, coagulum; *ep*, epiblast; *hy*, hypoblast. Other letters as in fig. 1.

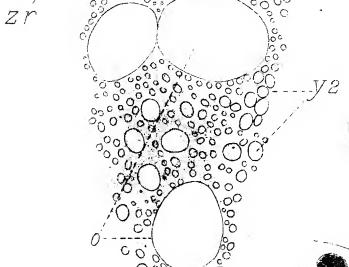
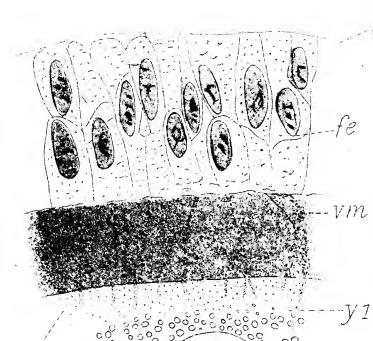


Fig. 1.

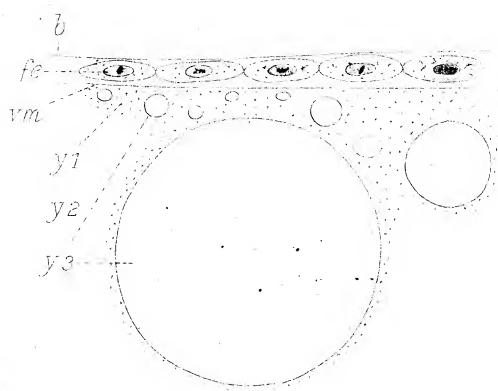


Fig. 2.

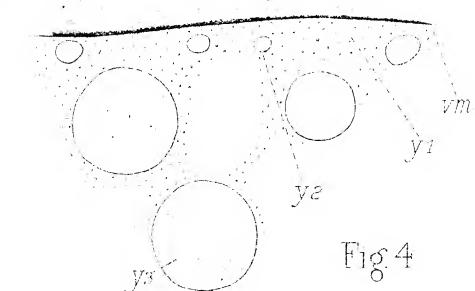
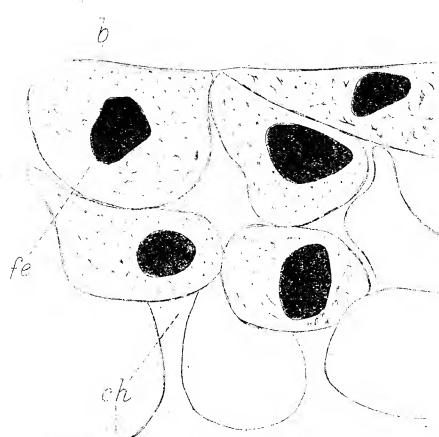
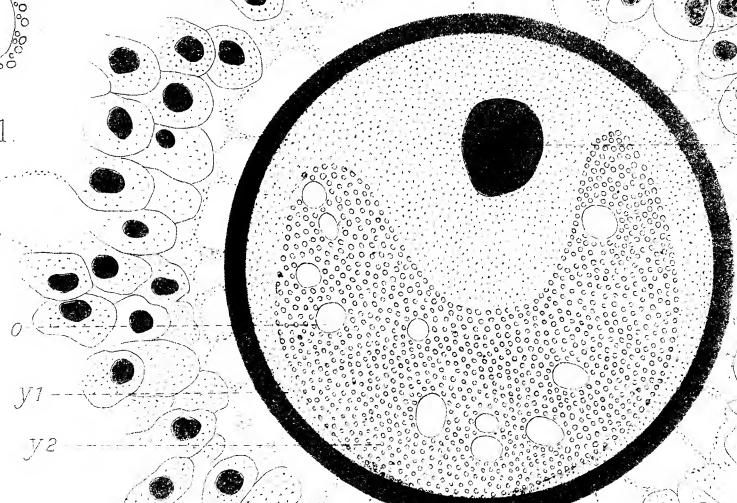
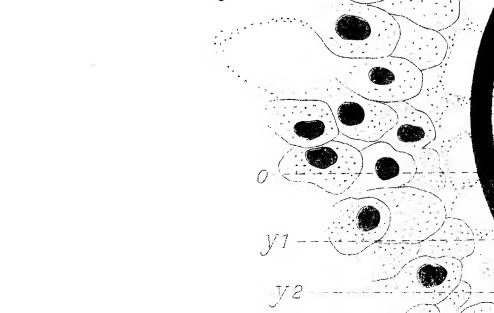


Fig. 4

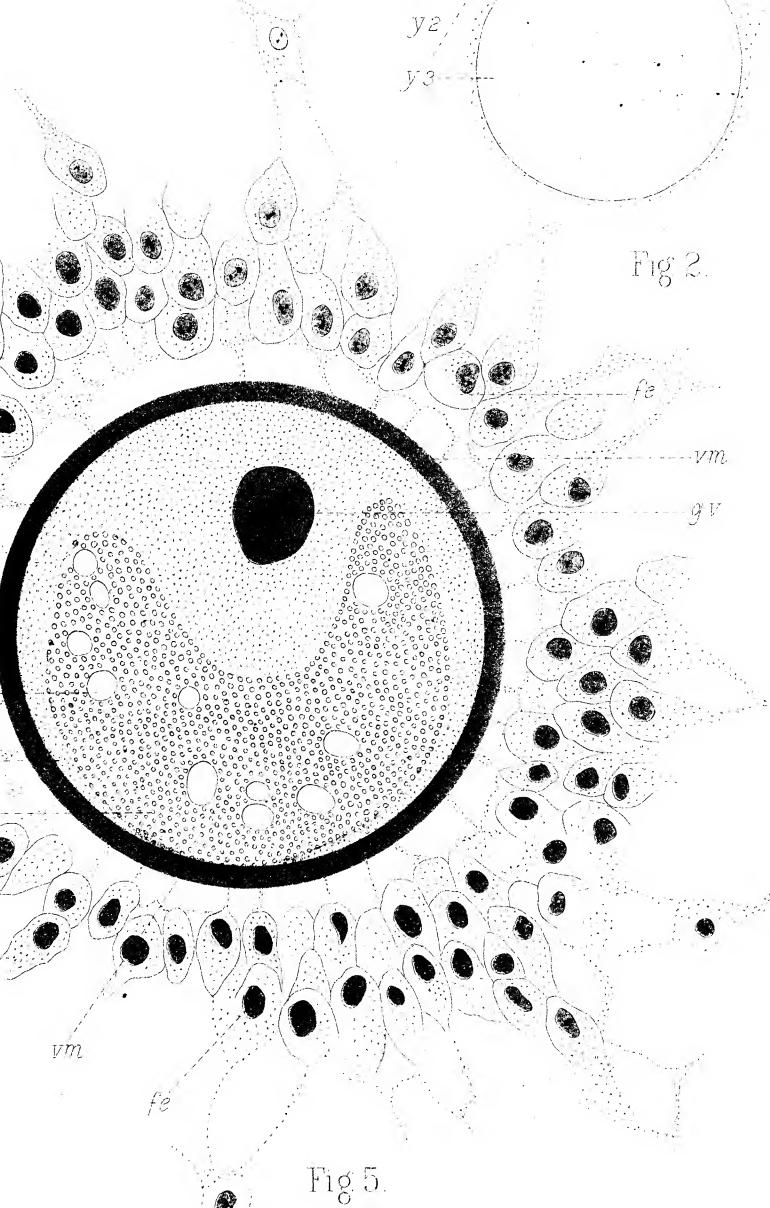


Fig. 5.

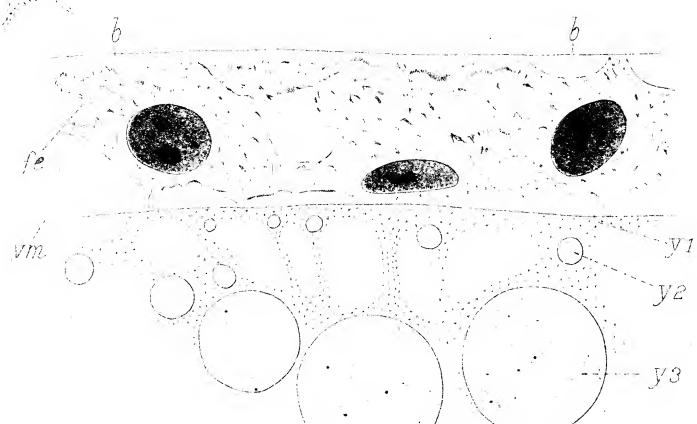


Fig. 3.

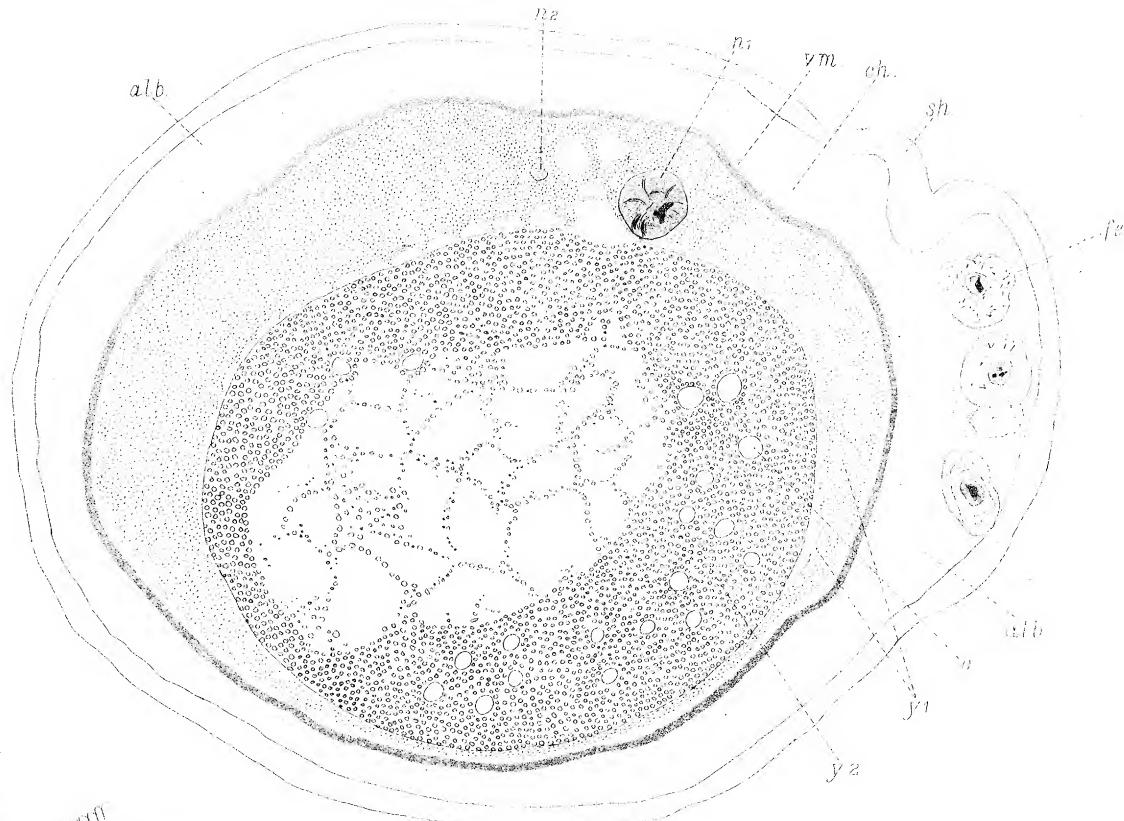


Fig. 5.

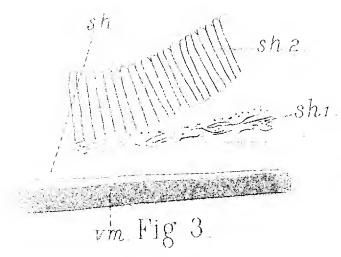


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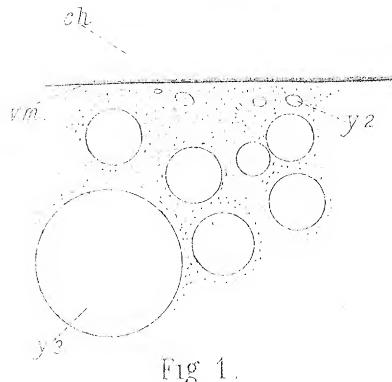


Fig. 1.

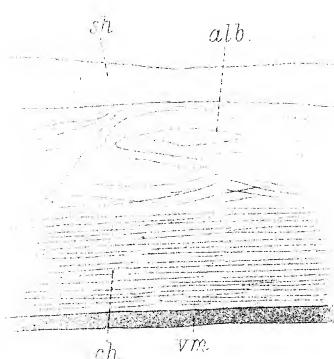


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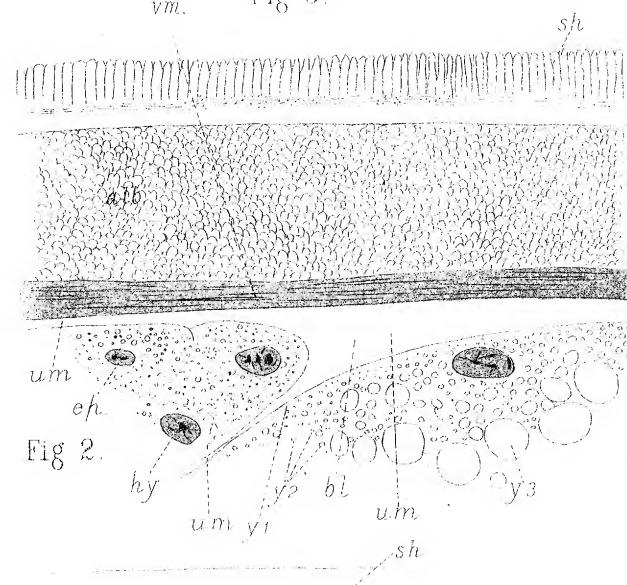


Fig. 2.

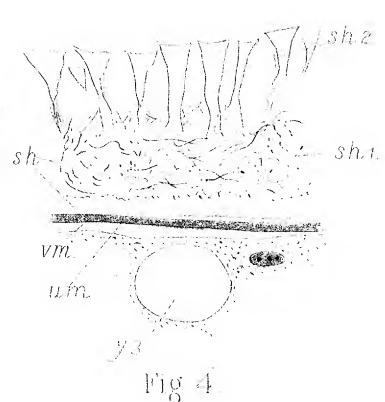


Fig. 4.

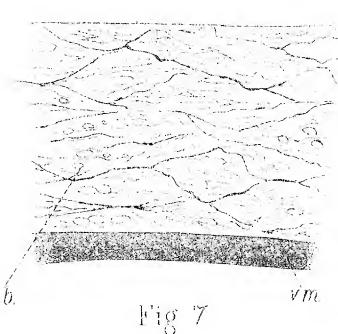


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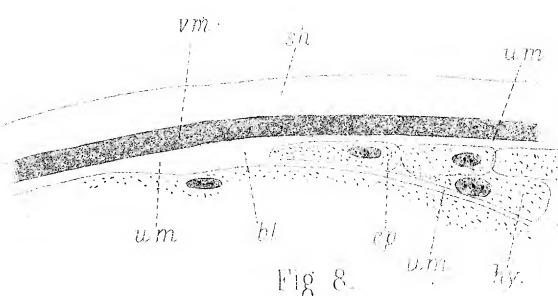


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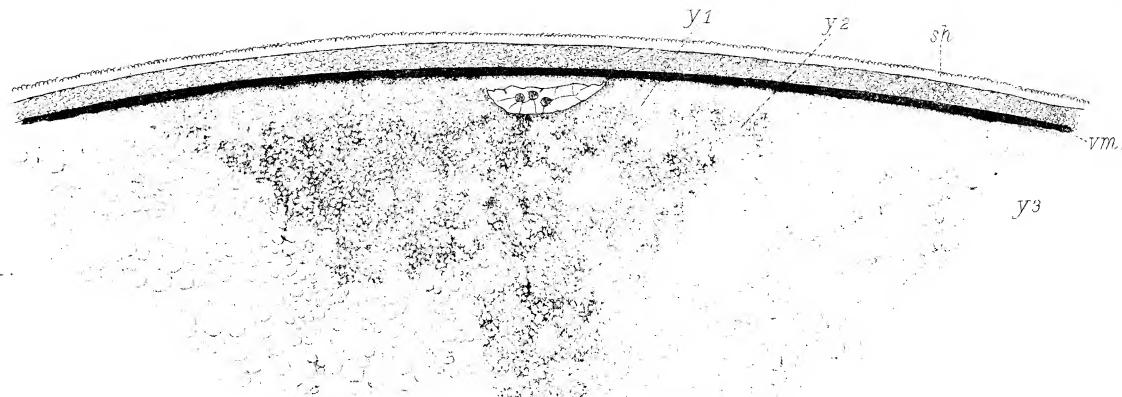


Fig. 1.



Fig. 2.

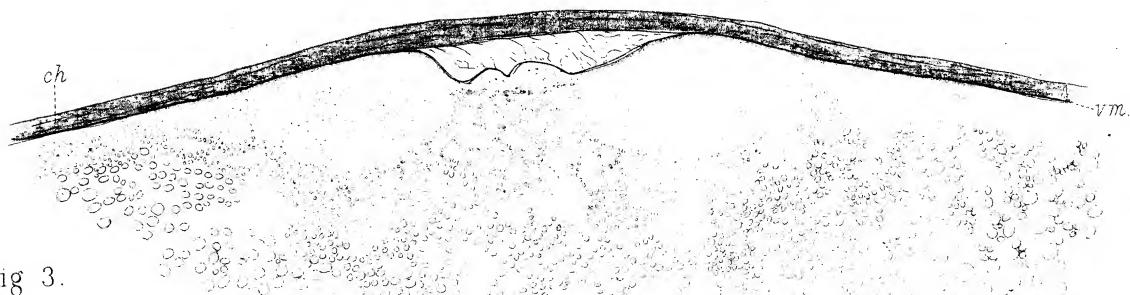


Fig. 3.

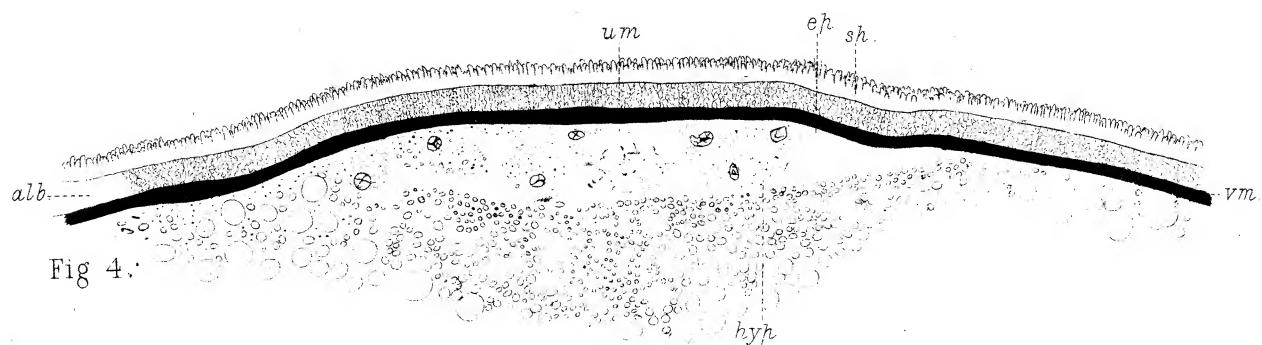


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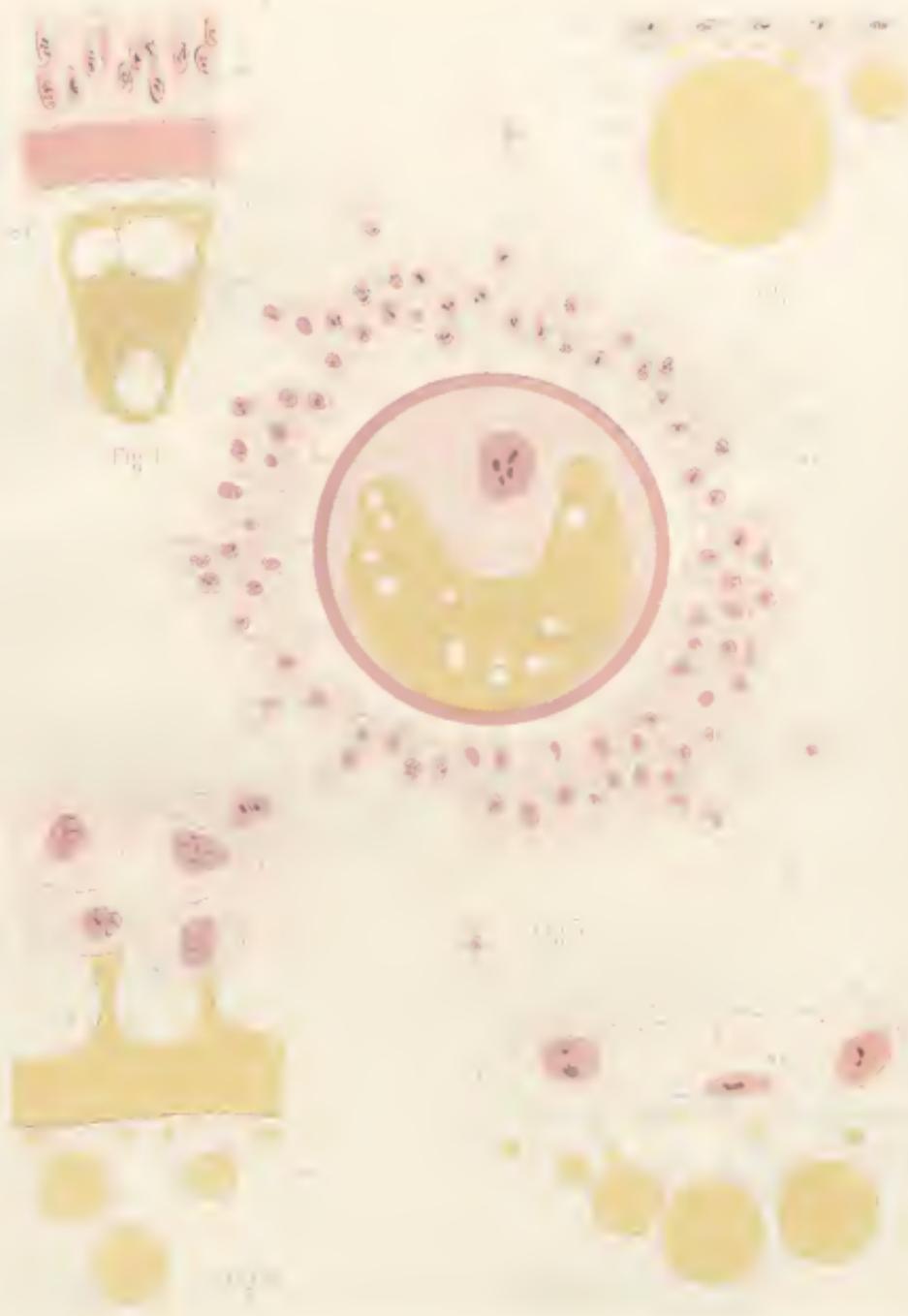


PLATE 29. ZEISS, oc. 2, obj. $\frac{1}{8}$ homogen. : cam. luc.

Fig. 1. *Echidna*.—Small portion of a section through the ovarian ovum, measuring 32 mm. in diameter. 1st period: *fe*, follicular epithelium; *vm*, vitelline membrane; *zr*, zona radiata; *y*, yolk granules; *yo*, white yolk; *o*, oil globule; *b*, basilar membrane.

Fig. 2. *Echidna*.—Small portion of a section through the ovarian ovum, measuring 1 mm. in diameter. 2nd period: lettering as in fig. 1.

Fig. 3. *Echidna*.—Small portion of a section through the nearly mature ovarian ovum. Beginning of 3rd period: lettering as in fig. 1.

Fig. 4. *Echidna*.—Small portion of a section through the ripe ovarian ovum, measuring 3 mm. in diameter. 3rd period: *ch*, pro-albumen.

Fig. 5. *Phascolarctos cinereus*.—Medium section through a nearly mature ovarian ovum taken from the "liquor folliculi" of a follicle measuring 9 mm. x 6 mm.: *fe*, follicular epithelium; *vm*, vitelline membrane; *gv*, germinal vesicle.

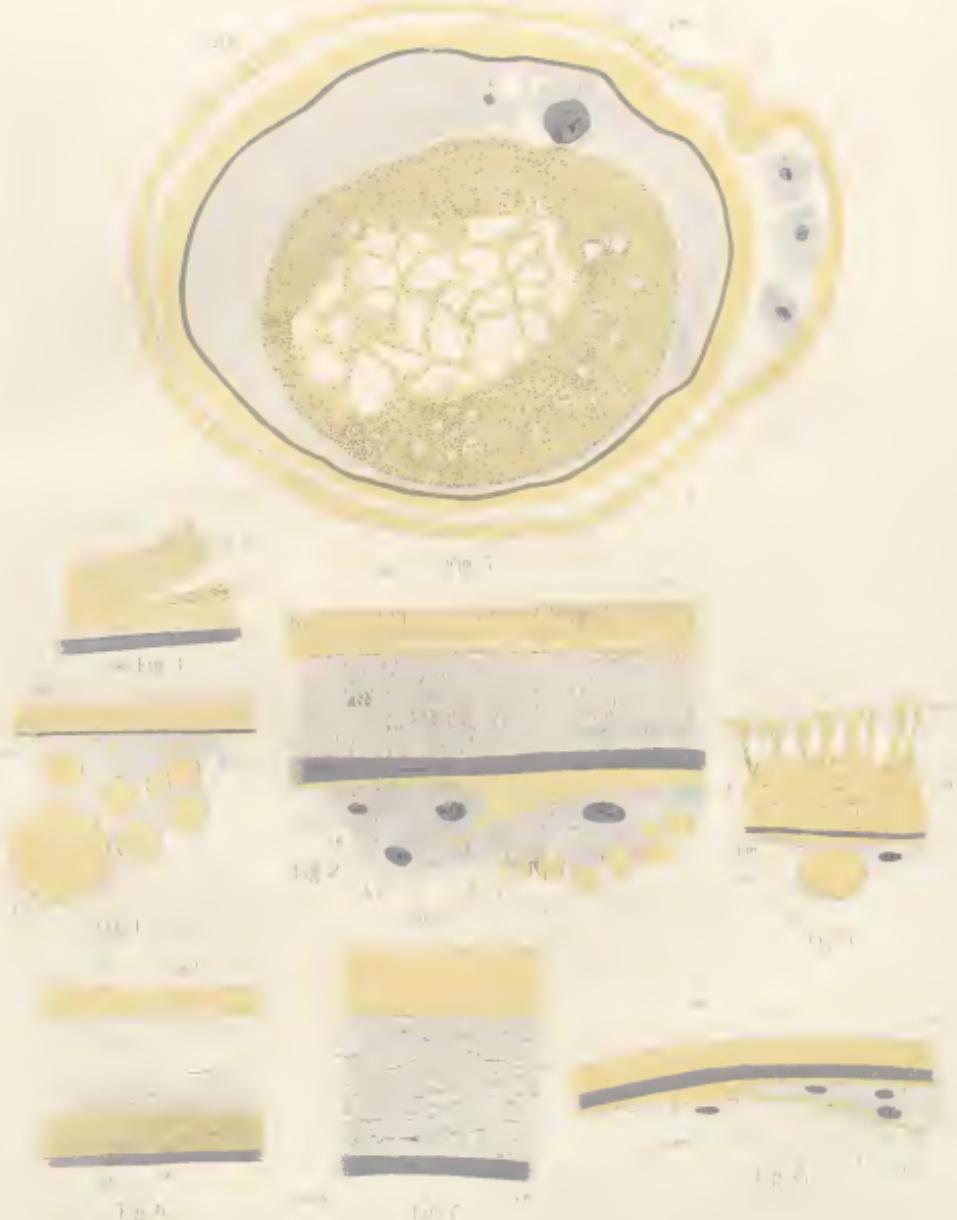


PLATE 30. ZEISS, oc. 2, obj. $\frac{1}{15}$ homogen. : cam. luc.

Fig. 1. *Ornithorhynchus*.—Small portion of a section through the segmenting ovum, taken from the open end of the Fallopian tube, measuring 2.6 mm. in diameter: *sh*, pro-albumen; *vm*, vitelline membrane.

Fig. 2. *Echidna*.—Small portion of a section through the segmenting ovum taken from the uterus, and measuring 6 mm. in diameter: *sh*, shell; *alb*, albumen; *vm*, vitelline membrane; *um*, coagulum; *bl*, blastopore; *ep*, epiblast; *hy*, hypoblast.

Fig. 3. *Ornithorhynchus*.—Small portion of a section through the segmenting ovum taken from the uterus, and measuring 6 mm. in diameter: *sh*, base of shell; *sh*₁, middle layer of ditto; *sh*₂, papillae of ditto.

Fig. 4. *Echidna*.—Small portion of a section through the blastodermic vesicle, taken from the uterus, and measuring 9 mm. in diameter: *sh*₂, cones derived from papillae of previous stage.

Fig. 5. *Phascolarctos cinereus*.—The 17th section of a vertical longitudinal series of 35 sections through the segmenting ovum, containing 2 nuclei, taken from the uterus, and measuring 1.7 mm. in diameter: *sh*, shell membrane; *fe*, cells of follicular epithelium; *alb*, albumen; *ch*, pro-albumen; *vm*, vitelline membrane; *y₁*, protoplasm, with finest yolk granules; *y₂*, white yolk; *n*, nucleus of smaller segmentation area; *n₂*, nucleus of larger segmentation area.

Fig. 6. *Phascolarctos*.—From uterus, 2.8 mm. in diameter, stage of 4 nuclei: *sh*, shell; *ch*, pro-albumen; *alb*, albumen; *vm*, vitelline membrane.

Fig. 7. *Phascolarctos*.—From uterus, 3.1 mm. in diameter; lettering as in fig. 6.

Fig. 8. *Hypsiprymnus*.—From uterus, 4 mm. in diameter: *sh*, shell membrane; *vm*, vitelline membrane; *um*, coagulum; *bl*, blastopore; *ep*, epiblast; *hy*, hypoblast. (Cf. fig. 2.)



PLATE 31. ZEISS, OC. 2, obj. c. : cam. luc.

Four sections through the germinal disc of *Echidna* and *Ornithorhynchus*. The violet corresponds to the distribution of the more active protoplasm. The yellow yolk spheres are coloured buff.

Fig. 1. *Echidna*.—Median section through the germinal disc of an unsegmented ovum taken from the lower part of the Fallopian tube, measuring 3·2 mm. in diameter: *sh*, shell; *alb*, albumen; *vm*, vitelline membrane.

Fig. 2. *Echidna*.—Stage of four segmentation nuclei. Lateral section through one pair of nuclei made in a plane at right angles to the first furrow; ovum 4·5 mm. in diameter: *n₁*, nucleus of smaller area; *n₂*, nucleus of larger area. Other letters as in fig. 1.

Fig. 3. *Ornithorhynchus*.—From open end of Fallopian tube, 2·6 mm. diameter. Stage of eight segmentation nuclei: *ch*, pro-albumen.

Fig. 4. *Echidna*.—From uterus, 5 mm. in diameter. Median vertical longitudinal section through germinal disc: *vm*, coagulum; *ep*, epiblast; *hy*, hypoblast. Other letters as in fig. 1.